On Cristispira veneris nov. spec., and the Affinities and Classification of Spirochæts.

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With Plate 20 and 2 Text-figures.

Contents.

Introduction.

During the last few years many memoirs have made their appearance in connection with the remarkable group of Protista which may be conveniently collected under the common name "Spirochæts." Of these organisms the most divergent descriptions have been given, and consequently the most divergent views have been held regarding their affinities with other organisms. Many workers consider that

the Spirochæts are allied to the flagellate Protozoa; many consider that their proper systematic position is among the Bacteria. As I have devoted a considerable amount of study to both these groups—as regards their cytology and life-histories—I have naturally been anxious to extend my studies to the Spirochæts. The present paper represents a part of the researches which I have made upon these organisms, with the conclusions derived from them. I may state at the outset that my own observations have led me to believe that the Spirochæts are really neither Protozoa nor Bacteria, but a group of Protista which—for the present—must be held to stand apart.

In the present paper I shall describe some researches which I made upon the Spirochæts of Molluscs—to one species of which I have devoted special attention. With the exception of the work of Schellack (1909) and Gross (1910), almost all the observations which have been made upon these forms are, I believe, marred by incorrect interpretation. My own observations and interpretations—made quite independently, and upon different material—correspond in many ways with those of Schellack and Gross.

I shall adopt the generic name Cristispira Gross to denote the flexible, spiral organisms which occur in the crystalline styles of so many Lamellibranchs. It is obvious that the name "Spirochæta," which has now for some time been applied to them, is no longer applicable (see p. 534).

Material and Methods.

The organism with which the present paper is chiefly concerned is a large species of Cristispira which inhabits the crystalline style of Venus (Meretrix) casta Chem. As no Cristispira has previously been recorded from this mollusc, I propose to name the new organism Cristispira veneris n. sp.

The discovery of this organism is due to Dr. Arthur Willey, F.R.S., who called my attention to it when I was

visiting Ceylon in 1909, during my tenure of the Balfour Studentship of Cambridge University (cf. Dobell, 1910). As the organism is of large size, I took the opportunity of investigating its structure and life-history as far as possible.

All the specimens of Venus casta which I examined were taken from Tamblegam Lake,[1] in the Eastern Province of Ceylon. These molluscs—together with others—were collected for me by Dr. Willey, at Niroddumunai, and sent thence to me in Trincomalee—about eight miles distant. With Dr. Willey's assistance I also examined some of the molluscs at Niroddumunai soon after they had been captured. I take this opportunity of again thanking Dr. Willey for his kind collaboration.

A crystalline style was present in 50 per cent. of the specimens of Venus casta which I examined. In every instance in which a style was present it was found to be infected with Cristispiræ. Examination of the contents of the œsophagus and stomach of individuals possessing no crystalline style was in every case negative; but only a few of these individuals were carefully examined. The Cristispiræ were usually present in large numbers, and were always very actively motile when first removed from their host.

After studying the living organisms, I made a number of permanent preparations in order to investigate finer details of structure. The method employed was the same as that which I have frequently used with success in studying similar forms, Bacteria and blood-inhabiting Protozoa. I made moist films of the substance of the crystalline style, fixed them by exposure to osmic vapour followed by absolute alcohol, and then stained them by Giemsa's or Leishman's modification of Romanowski's stain. The films were then washed in water and allowed to dry, or previously differentiated in weak alcohol. They were examined under an immersion in cedar-wood oil. This method gives, I believe, very accurate results when properly employed. The fixation with osmic vapour

[1] Tamblegam Lake is a salt-water lake, connected with Koddiyar Bay. All my observations were made in September, 1909.

must be done with care; if this is the case, harmful effects do not result from subsequent drying after staining. Minchin[1] has found a similar method suitable for studying the structure of trypanosomes. I have also obtained excellent preparations of these and hæmogregarines, etc., in this way.

I also made dry film preparations, fixed in absolute alcohol and stained by Giemsa's method in the usual way. This method gives rise to most misleading appearances in the organisms, but these are of considerable interest for comparison with those in other preparations which have been properly fixed.

The appearances observable in different preparations will be considered later, but it may be noted here that they vary according to the length of time during which the osmic vapour is allowed to act, and according to the degree to which the stain is extracted with alcohol after staining.

During my stay in Trincomalee, I was unable to use other methods of fixation and staining. But from my previous experience of the method I employed, I believe that my preparations are trustworthy, and give reliable information regarding the structure of the organisms. Comparison of my results with those of others has served to strengthen this opinion.

In all cases the films were made from the crystalline style immediately after removal. Cristispiræ usually undergo degenerative changes soon after they have been removed from their host, and therefore exhibit a structure which is very different from that of normal individuals. It is true that they will often live in carefully made preparations for several hours. But their motility, as a rule, diminishes rapidly, and their internal and external structure becomes modified by degenerative changes.

[1] E. A. Minchin. "The Structure of Trypanosoma lewisi in Relation to Microscopical Technique." 'Quart. Journ. Micr. Sci.,' vol. 53. 1909. p. 755.

Occurrence of Micro-Organisms in the Crystalline Styles of Lamellibranchs.

In addition to making an examination of the crystalline style of Venus casta, I searched for Cristispiræ in the styles of eight other species of Lamellibranch. All these were also obtained from Tamblegam Lake. I found Cristispiræ present in the style of only a single species—Soletellina acuminata Desh. Only three individuals out of eleven examined harboured the parasites, though a crystalline style was present in every individual. In one style, all the Cristispiræ were dead and degenerating when I found them.

These Cristispiræ in the style of Soletellina acuminata had been previously discovered by Dr. Willey (cf. Dobell, 1910). On account of the small amount of material which I obtained, I was unable to make any extensive observations upon these organisms. The Cristispiræ of this species are small, and resemble C. interrogationis Gross. I found similar forms—possibly identical with these—sometimes inhabiting the style of Venus casta, in company with the large C. veneris. As they were found in relatively small numbers I have not been able to make a careful study of them. I believe, however, that there can be little doubt that they belong to a separate species, and are not developmental forms of Cristispira veneris. The occurrence of more than one species of Cristispira in the same style has already been described by Schellack (1909) in several Lamellibranchs (Ostrea, Tapes, etc.), and by Gross (1910) in Pecten.

In some of the other molluscs which I examined, I found that the crystalline style was infected with Bacteria. These were not present simply as a few organisms—derived from the gut contents—on the surface of the style, but permeated the whole of its substance. In fact, the whole style appeared to be a pure culture of the particular organism which was inhabiting it. As far as I am aware, this has not been

observed previously in the styles of other Lamellibranchs, and I will therefore devote a few words to a description of my observations.

Out of nine individuals of Circe gibbia Lam. which I examined, five possessed a crystalline style, and three of these were heavily infected with Bacteria—two being uninfected. The Bacteria all appeared to be of the same species. They were non-motile Vibrio-like organisms of small size, and many dividing forms were present.

A single individual of Cyrena impressa Desh. which I examined contained a style heavily infected with a Bacillus.

Seven individuals belonging to the species Psammotæa variegata Wood were found to possess crystalline styles. Five of these contained large numbers of a Bacillus.

I examined ten specimens of Arca (Scapharca) rhombea Born, and found a style present in six of these. Four out of these six styles were filled with curious branching filaments, whose nature was not determined. I found the same sort of filaments in the style of one specimen of Soletellina acuminata. In the living state, the filaments look like fungal growths, and after staining by Giemsa's method they are seen to contain a large number of deeply staining granules. Owing to an unfortunate accident—a heavy thunderstorm which overtook me when I was returning to Trincomalee with my preparations—my slides of these organisms were much damaged, so that I can give no further particulars regarding these peculiar growths.

The window-pane oyster (Placuna placenta), of which I examined a few specimens, was always found to possess a very long and well-developed style. No parasites were found inhabiting it. Dr. Willey has also examined a number of styles of this mollusc, and always with the same negative results.

I give the results of my examination of the eight species of Lamellibranch referred to in the following table :

Mollusc.	No. of individuals examined.	No. in which a style was present.	Observations.
Arca (Scapharca) rhombea Born.	10	6	4 styles were infected with filaments: 2 uninfected.
Circe gibbia Lam.	9	5	3 styles were infected with Bacteria; 2 uninfected.
Cyrena impressa Desh.	1	1	Style infected with Bacteria
Placuna placenta L.	3	3	All styles uninfected.
Psammotæa variegata Wood	7	7	5 styles infected with Bacteria: 2 uninfected.
Solen (Ensis) regularis Dunk.	1	0	—
Soletellina acuminata Desh.	11	11	2 styles infected with a small Cristispira; 1 with dead Cristispira; 1 with filaments.
Venus (Dosinia) cretacea Reeve	2	1	Style uninfected.

I take this opportunity of thanking the Rev. A. H. Cooke for very kindly identifying these Lamellibranchs for me.

A good deal has already been written about the function of the crystalline style of the Lamellibranchiata. It has been suggested that it is a body of a secretory or excretory nature, that it is a reserve supply of food material, and that it is a mechanical device for catching and conglomerating food particles. Mitra[1] has shown that it contains a proteid substance—which he showed to be a globulin—and that an amylolytic ferment is present in it. He therefore regards it as a body which is primarily connected with the digestion of

[1] Mitra, "The Crystalline Style of Lamellibranchia," 'Quart. Journ. Micr. Sci.,' vol. 44, 1901.

food. Hornell,[1] from his own observations on the style of the oyster, regards the style as a food-catching apparatus, as was maintained earlier by Barrois. Pelseneer[2] states that "the product of its solution forms a sort of cement which encrusts any hard substances that may have been ingested and thus protects the delicate walls of the intestine from injury."

This is not the place to discuss these and other views which have been put forward regarding the functions of the crystalline style. But as this is of some importance in connection with the organisms which inhabit it, the structure itself cannot be ignored. It appears to me most probable—from the observations recorded by others—that the crystalline style serves both to catch food particles and prepare them mechanically for digestion and also to assist in the digestion of the amyloid constituents of these particles.

In some Lamellibranchs—e. g. in Pecten (Gross, 1910)—the Cristispiræ are found in the stomach and intestine, and only rarely in the crystalline style. It therefore seems to me probable that Cristispira is really a gut parasite, which often happens to find the substance of the crystalline style a suitable culture medium. The same is also suggested by the occurrence of Bacteria in the style. The latter contains some 12 per cent. of globulin, with about 1 per cent. of salts and 88 per cent. water.[3] It might therefore well serve as a culture medium for many micro-organisms which reach it accidentally. I do not think any deeper significance need be attached to the association of Protista with the crystalline style.

Cristispira veneris, n. sp.

I will now record my observations upon the structure

[1] Hornell, "Report on the Operations on the Pearl Banks during the Fishery of 1905," 'Ceylon Marine Biological Reports,' Part II, June, 1906.

[2] Pelseneer, "Mollusca," in Lankester's 'Treatise on Zoology,' London, 1906.

[3] Mitra, loc. cit.

and mode of division of Cristispira veneris—the large "mollusc spirochæt" which I found inhabiting the crystalline style of Venus (Meretrix) casta Chem. in Tamblegam Lake. I shall here give my own observations only—reserving an analysis of my own results and those of other workers for the next section (p. 527).

(1) Structure.

Cristispira veneris is one of the largest members of the genus, resembling C. balbianii Certes and C. pectinis Gross. The average length is 50–60 μ, the average breadth—in fixed and stained specimens—about 1·5 μ. A certain amount of variation in the breadth of different individuals is observable in fixed and stained organisms—the narrowest being slightly over 1 μ, the broadest approximately 1·9 μ. Dried films stained with Giemsa not uncommonly possess a width of almost 2 μ. The longest undivided individual which I have measured was 74 μ in length.

Living individuals appear to be of approximately the same width, though it is almost impossible to make accurate measurements of them on account of their great motility. The differences in width observable in stained individuals are due, I believe, to the greater or less degree of flattening which takes place in the organisms in making the preparations. It can be seen in the living organisms that they are cylindrical—that is to say, they are circular and not band-like in optical transverse section. In the process of making films, the cylindrical shape is modified by flattening to a band-like shape, thus making the individuals appear broader. Thus, if the diameter of the cylinder constituting the organism were 1 μ, the circumference would be $\frac{22}{7}$ μ. If complete flattening of the cylinder occurred, the breadth of the organism would appear to be $\frac{1}{2} \times \frac{22}{7}$ or approximately 1·6 μ. According to the amount of flattening which occurred, different individuals

might therefore display any breadth between 1 μ and 1·6 μ. If the breadth of C. veneris is therefore a little more than 1 μ—that is, about 1·2 μ, subject of course to slight individual variation—then the different breadths observed in stained specimens are easily accounted for by the different degrees of flattening which different individuals have undergone in the process of making the preparations. I believe, therefore, that the body of C. veneris is cylindrical, and has an actual uniform diameter of approximately 1·2 μ in the living organism.

I have already described a similar apparent variation in breadth—due, I believe, to the same causes—in the case of Bacteria (see Dobell, 1910 A). The apparent variability in the breadth of different individuals of Cristispira is a point of some importance when considered in relation to the method of division (see p. 526).

As in other members of the genus, the body of C. veneris possesses a spiral, corkscrew-like shape. The number of complete turns in a full-grown individual is approximately four. The number is greater than this in dividing individuals (five or six), and less in newly divided individuals (two or three).

In the living organisms, I have not been able to distinguish any structure in the protoplasm of the cell, which appears homogeneous under the highest magnification which I was able to employ (Leitz $\frac{1}{12}$ in. oil-immersion × ocular 5, using direct sunlight for illumination). A few small refractile granular inclusions were usually to be seen in the protoplasm.

The ends of the organism are bluntly pointed (see fig. 1, Pl. 20), being less rounded than the ends of C. balbianii and less pointed than those of C. anodontæ. The body usually tapers very slightly towards the two ends. The structures called "polar caps," described in C. balbianii, C. pectinis, etc., are not observable in C. veneris. They appear to be confined to the species which possess rounded ends.

Neither in living nor in fixed and stained specimens can any structures comparable with flagella be seen.

Like other Cristispiræ, C. veneris has a flexible body. It may be noted, however, that in living and actively moving individuals the body is kept relatively rigid—flexibility being chiefly observed in slowly moving (? abnormal) individuals, and indicated by the irregular spiral conformation often observable in fixed and stained organisms. I believe that bending movements occur very seldom in normal active individuals. The ordinary movements of C. veneris are similar to those of C. balbianii, which have already been described by Perrin (1906).

The two most important characteristics of the Cristispiræ are the crista and the structure of the protoplasm. I will now describe these in detail in C. veneris.

The Crista.—This structure, formerly called the "undulating membrane" on account of its supposed homology with the undulating membrane of trypanosomes, has hitherto been correctly interpreted—I believe—by Gross alone. The name crista, or crest, which he has proposed for it, appears to me a convenient and suitable one. I shall therefore adopt it.

A crista is present in every individual which I have examined in the living condition or in properly fixed and stained preparations. In dried Giemsa preparations, it may be torn and distorted and sometimes appear completely lacking, but this is due to the drying which has taken place before fixation, and is therefore not a normal condition. In all cases in which proper fixation with osmic vapour has been effected, the crista is present and presents the same characteristic appearance.

The crista is in the form of a narrow band, radially situate on the surface of the organism, and spirally disposed (see fig. 2). It does not as a rule reach the extreme ends of the organism, and appears to me to be a simple prolongation of the membrane which clothes the body. At the ends it merges gradually into this, and no structures comparable with basal granules or blepharoplasts are present. It is homogeneous

throughout, and shows no fibrillar structure in living or properly fixed specimens. It is stained a pink or violet colour by Giemsa's method, in marked contrast with the general blue colour of the body (see fig. 2, etc.). There is no thickened, chromatic edge to the crista. In fact, it does not in any way resemble the undulating membrane of a trypanosome.

In macerated individuals the crista may present a very different appearance (fig. 3). It becomes greatly enlarged and distorted, and shows a very definite fibrillar structure. This is an artifact, and though it may indicate that the crista is really composed of fibrils arranged longitudinally, it must not be forgotten that in normal individuals it appears absolutely homogeneous. This fibrillar appearance has often been described as the normal structure of the "undulating membrane" of Cristispira—which it certainly is not.

The crista of C. veneris is therefore a delicate, uniform, band-like appendage, wound spirally round the body, and extending almost to the ends. It is always present, and has no resemblance to the undulating membrane of a trypanosome. It serves, apparently, as a rigid lateral fin-like extension of the body, in the performance of the screw-like movements of the organism. Some further account of some of the previous interpretations of this structure will be found on p. 528.

Structure of the Protoplasm.—As I have already noted, the protoplasm of the living organisms appears homogeneous. In stained specimens, however, it has a distinct and highly characteristic structure. This structure has been observed by Schellack and Gross, though the interpretations of these two observers differ.

If a Cristispira be fixed by exposure to osmic vapour for about thirty seconds, then transferred immediately (without any drying being allowed to take place) to absolute alcohol for ten minutes, then stained by Giemsa's method, and examined in the manner already described (p. 509), it is seen to possess a structure like that of the individual shown in fig.

I, Pl. 20. This organism is from a moist film preparation of a crystalline style which was so treated. The whole organism (fig. 1) shows a protoplasmic structure consisting of a single row of chambers or alveoli. The walls of these chambers are stained a deep blue, their contents a uniform pale blue. The relative dimensions of these chambers are not always constant; they may vary not only in different organisms, but at different points in the same organism—being sometimes square, sometimes oblong (cf. figs. 7, 8, etc.). The alveolar walls separating adjacent chambers from one another appear as transverse septa in optical section (see figs. 1, 2, etc.). At the point where the transverse septum joins the wall of the cell a dark purple granule can be seen. The whole organism thus appears to contain a series of paired purple granules, united by blue transverse lines—representing the alveolar walls (fig. 1). This appearance is always presented by individuals treated in the manner described. If the exposure to osmic vapour has been limited to about thirty seconds, and no overstaining has taken place, then the appearances are constantly encountered. The difference in size observable in the purple granules should be noted—also the fact that they always lie at the edges of the organism, and never centrally (figs. 1, 7).

If the osmic vapour be allowed to act for a longer period of time—i. e. for several minutes—then the organisms present a different appearance after Giemsa staining. The granules appear much smaller, and are stained a deep blue (figs. 2, 8). The chambers are easily visible, but the granules have dwindled to tiny dark blue points. In some cases they cannot be distinguished with precision at all levels in the body (cf. fig. 6).

Organisms which have been dried previous to fixation, fixed in absolute alcohol, and then stained by Giemsa's method in the usual way, often present appearances which are quite different from those seen in osmic-fixed organisms. They show, in fact, all the remarkable "chromatin" configurations which have been described by Perrin and others. The

chambers are often indistinctly seen, or absent. Vacuoles are not infrequently present. Red "chromatin" structures of varying form are seen in different individuals and at different points in the same individual. Fig. 10 shows some of the "nuclear" structures observable in dried organisms. It is drawn from a part of a Cristispira which was dried before fixation, fixed in absolute alcohol, and stained by Giemsa's method. It will be seen that the "chromatin" is in the form of spiral or zig-zag filaments, rods, granules, "tetrads," etc. These arrangements of the "chromatin" are found side by side in the same organism at the same time.

The appearances which are observable in organisms which have undergone plasmoptysis are instructive. Such an individual is shown in fig. 3. The whole organism is filled with red granules, of variable size and irregular distribution. At the points where the cell membrane has burst, the protoplasm has flowed out, and it can be seen that it consists of two different substances—a bluish or lilac coloured substance and a denser dark-red substance.

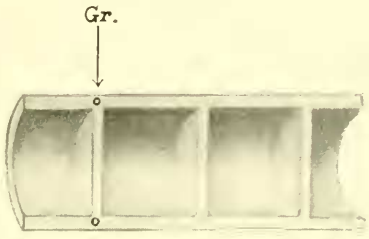
How are all these different appearances to be interpreted? I believe the correct interpretation is as follows: The structure of Cristispira may be compared with that of a bamboo stem. The whole body is in the form of a hollow cylinder, divided into a single series of chambers by means of a series of transverse disc-like partitions like the nodes of a bamboo rod. The cytoplasm forming the walls of the cylinder and the disc-like partitions is dense and deeply stainable; the cytoplasm which fills the chambers is less dense and less deeply stainable. Text-fig. 1 illustrates diagrammatically the structure of a portion of a Cristispira which is supposed to have been split longitudinally, so as to divide the body into two equal parts. When viewed from inside, an appearance such as is shown in Text-fig. 1 would be seen.

The tube forming the body is divided into cylindrical chambers by transverse disc-like partitions—only half of each disc and chamber being seen, of course, when the other half is split off. In a Cristispira all the solid structures dia-

grammatically represented in Text-fig. 1 are composed of the denser part of the cytoplasm, the chambers—represented empty in the diagram—being filled with the less dense cytoplasmic matter.

Now I believe that the only other morphological con-

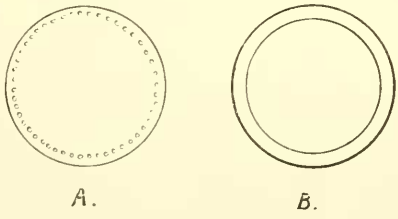
TEXT-FIG. 1.



Explanation in text.

stituents of the cell are a number of small granules, which are arranged round the circumference of the disc-like partitions—in the dense cytoplasm which lines the cell. The position of these granules—which I suppose to form a ring when a partition is seen in a transverse section of the whole

TEXT-FIG. 2.



Explanation in text.

cell—is shown in the diagram (text-fig. 1, *Gr.*). A transverse section of the cell, passing through a partition, would present an appearance similar to that shown in Text-fig. 2 A. In this diagram it will be seen that the granules are arranged in the form of a ring round the circumference of the disc. A

transverse section of a cell, passing through the middle of a chamber, would present an appearance like that shown diagrammatically in Text-fig. 2 B. The appearance is that of a tube—the wall composed of dense cytoplasm, the inside filled with less dense and more lightly staining cytoplasm.

Now I think that those individuals which have been exposed to osmic vapour for several minutes and then stained with Giemsa's stain, present appearances which must be interpreted as representing a structure such as I have just described. The chambered structure of the cytoplasm, with the ring of small granules round the circumference of each partition, is quite clearly seen in these individuals. In optical section, of course, only a single pair of granules is seen—lying at the point where the partition joins the cell wall. The granules are of very small size, and are therefore visible under the highest powers only, and after correct differentiation. It is difficult to be absolutely certain that a ring of granules is present round each partition, but I believe that this can often be demonstrated. As the bodies of *Cristispiræ* treated in this way remain cylindrical—or undergo only a very slight flattening—in the process of fixing and staining, it is necessary to suppose such an arrangement of the granules to account for their constant appearance at the edges of the organism—at the points where the septa and cell-walls unite (cf. figs. 2, 8, etc.).

As I have pointed out, the organisms which have been fixed by exposure for a shorter time to osmic vapour show pairs of much larger purple granules situated at the points where the partitions join the sides of the cell. A ring of granules is not present in these forms. I believe the correct interpretation of such organisms (figs. 1, 7) is as follows: In the course of making the preparation the organisms have become flattened, as a result of drying following upon inadequate fixation. Exposure to osmic vapour for about half a minute is not sufficient to fix the organisms properly. As they dry on the slide the granules run together into small masses at the edges of the organism, and so give rise to the appear-

ances which I have described. It is easy to understand why the granules—in reality masses of granules—appear to be of different sizes in such organisms (fig. 1), and why they always appear at the edges of the organism, which we know to be really cylindrical when alive.

When no fixation previous to drying occurs, the cell undergoes plasmolysis and complete flattening on the slide. The small granules run together in various ways, giving rise to the various "nuclear" figures which have been described (cf. fig. 10). It is easy to understand how the flowing of the granules through the walls of the chambers, and their massing together in various ways, can give rise to the appearance of transverse bars, spirals, tetrads, etc., of "chromatin." It will hardly be necessary to describe in detail the several ways in which such appearances may be caused.

The staining reactions of the substance of which the granules are composed require a brief consideration. I have already noted that the granules stain a deep blue after a long exposure to osmic vapour, purple after a brief exposure, red when osmic fixation is omitted, and only absolute alcohol is employed after previous drying. I believe these differences are directly due to the action of the osmic vapour—prolonged action of which so changes the granules that they are unable to take up the red-staining element in the Romanowski stain. I have observed this action of osmic acid in the case of Bacteria and many Protozoa, and I believe it must have been noticed by many other workers who employ Romanowski staining after osmic fixation. A short exposure to osmic vapour permits the granules to stain red—as they do when not acted upon by it. A longer exposure permits them to stain red to a less extent, and gives rise to a purple coloration. Still longer action of the osmic vapour renders the granules incapable of taking up the red element in the stain, and they therefore appear blue—the blue element alone being capable of staining.

There can be no doubt, I believe, that the granules are composed of a substance which is different from that of the

cytoplasm. It is a substance, moreover, which may be stained red with Giemsa's stain (cf. figs. 3, 10). From this it may perhaps be inferred that the granules are composed of a chromatin substance, and are therefore of a nuclear nature. This consideration, however, does not really justify the conclusion that the granules constitute the nuclear apparatus. Further evidence of the behaviour of the granules during other phases of the life-history is required before their true significance can be settled. Yet for the present, I regard the nuclear hypothesis as the most probable, and believe that the granules represent a chromidial nucleus somewhat similar to that which occurs in many Bacteria (see Dobell, 1910A), and some Protozoa.

To summarise my interpretation of the protoplasmic structure of Cristispira: The whole body is composed of a single series of cylindrical chambers or alveoli, separated from one another by disc-like partitions. These structures are composed of a denser cytoplasm constituting their walls, and a less dense cytoplasm which fills the chambers. Very small granules—probably constituting, as a whole, a nucleus of a chromidial form—are arranged round the circumference of each disc-like partition. Various appearances—such as a series of pairs of large granules, tetrads, transverse bars, spiral filaments, etc., of chromatin—which are often encountered, and have been frequently described by others, are artifacts.

One more point in the protoplasmic structure of Cristispira veneris requires consideration. It often happens that here and there, in the body of an individual, certain chambers appear more darkly stained than the remainder. This appearance is well seen at the point marked *a* in the individual depicted in fig. 6. At other times the partitions between the chambers appear thickened (see fig. 8, *b*, etc.), and appearances which are intermediate between a darker chamber and a thickened septum are also to be seen (see fig. 9, where this is shown in two places). Similar appearances have been figured by Gross and others.

The explanation of these appearances is, I believe, quite simple. As will be shown in the next section of this paper (vide infra), the method of multiplication is by transverse fission. The daughter-individuals which arise from the transverse division of a long individual are therefore short—being only half the length of the original organism. Before they undergo a subsequent division they must grow in length, and must therefore form new chambers. I believe that these new chambers are formed at various points in the body, and arise by the gradual thickening of a partition and its subsequent hollowing out. Thickened partitions therefore correspond to the points where new chambers are beginning to be formed—more darkly stained chambers are newly formed chambers. Successive stages in the formation of chambers in this way are shown in fig. 8 (where a thickened septum is seen at *b*), fig. 9 (which shows the hollowing of the septa at two points), and fig. 6—where a darkly staining (newly formed) chamber is seen at *a*.

(2) Division.

Although I have not been able to observe every stage in division in the living organism, I have encountered a number of dividing forms in my stained preparations which leave no room for doubt as to the essential features of the process. Division is transverse, and is effected in the manner described by Gross in the case of C. pectinis. I have never seen any indications of a longitudinal division, and all the observations which I have made speak strongly against the view that such a method of multiplication occurs in these organisms.

The long individuals which are about to divide into two transversely are in the form of spirals consisting of five or six complete turns. Before dividing, they bend themselves double—the two halves becoming intertwisted (see fig. 4). This phenomenon has been described in C. pectinis by Gross, who calls it "incurvation." The transverse fission of the organism begins when it is in this condition. It occurs

in the middle of the incurved individual, at a point where a transverse partition separates two adjacent protoplasmic chambers from one another (cf. fig. 4). The partially divided organism then untwists itself—passing out of the condition of incurvation to the original form of a simple spiral (fig. 6). In this condition fission is completed, and the two daughter-individuals separate from one another. The latter are, of course, short individuals in the form of spirals consisting of two or three turns.

In the division of the body the crista is also involved. It divides with the rest of the body, in the manner shown in fig. 7. This figure shows the middle region of a dividing Cristispira which is just straightening itself after being in the state of incurvation.

The whole process of division is extremely simple, and resembles—apart from the incurvation—the process of division which can be seen in many Spirilla and other Bacteria.

I think there can be no doubt at all that the incurved individuals are not really stages in a longitudinal division—as they seem frequently to have been interpreted by other workers. The crista does not split longitudinally. I have never seen partially longitudinally split individuals; the transverse division of the looped end of the incurved organism is often very easily seen; the number of turns in the spiral in a newly divided individual is half that of the undivided individual; and finally, the width of all individuals—when allowance is made for the differences due to technique (see p. 515)—is fairly constant. These facts indicate most clearly that division is transverse and not longitudinal, as Schellack and Gross have maintained in the case of other species of Cristispira. I believe, with these two observers, that all cases of longitudinal division which have been described in Cristispira are due to misinterpretation of the observed appearances.

Formation of gametes, conjugation and encystation I have never encountered. These phenomena—first described by

Perrin—have been said to occur by several observers, but their statements are based, I believe, upon a wrong interpretation of the facts. This has already been pointed out by Schellack and others, so I will therefore omit further discussion of the matter here.

The Morphology, Affinities and Classification of Spirochæts.

In the following pages I shall discuss the most important features in the morphology and life-history of the Cristispiræ, or, as they are commonly called, "mollusc Spirochæts." A discussion of these features is necessary in order to arrive at conclusions regarding the affinities of this remarkable group of organisms, and of Spirochæts in general.

Two excellent contributions to this subject have recently been made—that of Schellack (1909) and that of Gross (1910). Both these workers employed good cytological methods, and made careful detailed observations on the forms which they investigated. As they have both discussed the earlier work at some length, and entered fully into the literature on the subject, I will confine myself chiefly to pointing out wherein my results agree with or differ from those of these two workers.

The Cell Membrane.—The body of a Cristispira is bounded by a cuticle-like covering, which I shall call the cell membrane. This membrane is usually termed the "periplast"—a name originally applied to it by Perrin, who believed the organisms to be Trypanosomes. The use of this special word for the cuticular covering in these two groups of organisms—Spirochæts and Trypanosomes—appears to have led many people to believe that the cell membranes are so similar to one another, and different from other cell membranes, as to indicate affinities between the two groups. The only real similarity between the cell membrane of a Cristispira and that of a Trypanosoma is that the same word is used for both. Both are, of course, modified forms of mem-

brane which bound the protoplasm of the body; but such membranes are found in the majority of Protista, only they are not usually called "periplasts." I shall therefore avoid using this term, as I believe it leads to a confusion of ideas; and I shall speak of the cuticular covering of a Cristispira as the "cell membrane," or simply as "the membrane."

A membrane certainly exists in Cristispira. Unless this were present, it is difficult to see how the contours of the body are preserved. The appearance of burst individuals also indicates that a membrane of some sort is present (see fig. 3). Moreover, the presence of a membrane is clearly demonstrated when the organisms undergo plasmolysis. This has been clearly shown by Swellengrebel (1909) in C. balbianii.

It has frequently been stated that the "periplast" of Cristispira possesses a fibrillar structure, which can be seen when the organisms are macerated. I have seen many individuals of C. veneris which show the appearances which have been thus interpreted, and I believe the fibrils are derived in all cases from the crista (see fig. 3). The cell-membrane itself possesses no structure. Schellack (1909) states that "bei den grossen Spirochæten[1] ist ein fibrillärer Periplast sicher nachgewiesen; er kann künstlich aufgefasert werden." I believe this is incorrect. My own view is the same as that expressed by Gross—"Der Periplast existirt gar nicht. Die Cristispiren haben einfach eine ziemlich starke, aber färberisch nicht differenzirbare Zellmembran." As Gross has discussed the matter fully I will say nothing further about it—merely pointing out that my interpretation agrees with his.

The Crista.—Schellack (1909) interprets the crista as an artifact—"als ein durch künstliche Veränderung des Periplasts hervorgerufenes Gebilde." I believe this interpretation to be quite incorrect. The crista is easily visible in slowly moving, living organisms, and is constantly present in properly fixed specimens. It is homogeneous and possesses

[1] I.e. Cristispiræ.

no chromatic border. It is totally different from the undulating membrane of a Trypanosome, to which most previous workers have likened it. My interpretation of this characteristic structure is the same as that of Gross (1910). "Die Crista ist ein Organell sui generis."

A deeply staining ("chromatic") edge to the crista and a fibrillar structure can only be seen in macerated organisms, or organisms which have been imperfectly fixed. Such structures must therefore be regarded as artifacts. The normal crista of C. veneris stains pink or violet with Giemsa's stain, but this does not necessarily indicate that it contains chromatin.

Flagella.—Flagella or cilia, such as occur in flagellate Protozoa or Bacteria, are not present in Cristispiræ. The matter has been fully discussed by Schellack (1909) and Gross (1910), who have both come to this same conclusion. Further discussion will therefore be superfluous.

Protoplasmic Structure.—The chambered structrue of the protoplasm, which I have described in C. veneris, has already been clearly recognised in other Cristispiræ by Schellack and Gross. I am convinced, with these two observers, that the various nuclear figures (spiral filaments, transverse rodlets, tetrads, etc.) described by Perrin and others are really artifacts. Moreover, Perrin's account (1906) of the relations existing between the various nuclear figures and the longitudinal division of the organism must be discarded. For the nuclear figures are artifacts, and longitudinal division does not occur.

The interpretations of the appearances observed by Schellack and Gross differ from that which I have given in preceding pages. It will therefore be necessary to discuss their views briefly.

Schellack's (1909) interpretation of the protoplasmic structure of Cristispira is somewhat similar to mine. His description of the structure of the chambers is in close agreement with my own description. In one point, however, Schellack's interpretation differs from mine. He believes

that chromatin granules are scattered through all the walls of the chambers, whereas I believe that—in C. veneris—the granules are confined to the circumference of each transverse disc-like partition. Schellack thus regards a Cristispira as containing a nucleus of a kind of chromidial form.[1]

Gross's (1910) interpretation is peculiar. Although he appears to have observed the same structures as Schellack and myself, he comes to the conclusion that the protoplasm is really structureless, and there is no nucleus of any sort present. The chambers are artifacts, because they can be seen neither in the living organisms nor in organisms fixed with Flemming's fluid and stained with iron-hæmatoxylin. Gross always found the chambered structure present after fixation with corrosive sublimate, but he attributes this structure to the action of the fixative.

I believe that another explanation is correct. I believe that the invisibility of the chambered structure after fixation with Flemming's fluid is the direct result of the action of the fixative. It is often exceedingly difficult to obtain good differentiation of the internal structure of Bacteria after they have been fixed with Flemming's fluid, and I believe that this is due to the action of the fluid upon the cell-membrane and the protoplasm. Every cytologist must have experienced, at some time or other, a difficulty in staining cells after fixation in Flemming's fluid. At all events my own experience leads me to believe that this must be so. I would also point out that, in the case of C. veneris, not only does a prolonged action of osmic vapour—in the course of fixation—cause a change in the staining reactions of the granules, but it also gives

[1] This statement requires some qualification. For although Schellack describes the chromatin as being in the form of granules ("Die Kammerwände scheinen aus einer festeren Substanz zu bestehen und es sind ihnen Körnchen aufgelagert," p. 400), he seems inclined in another place to regard the nucleus as being constituted by the whole of the substance of the chamber walls. He says: "Die Gesamtheit der Waben in einer normalen Spirochæte bildet einen ziemlich fest in sich haltbaren, kompakten Stab, den sogenannten Kernstab. Die Periplasthülle liegt ihm direkt auf," etc. (p. 401).

rise to a less precise staining of the cell as a whole. Cristispiræ which have been subjected to osmic vapour for many minutes tend to take up a more diffuse blue stain, and show the chambered structure less distinctly in consequence. But although this is the case, the chambers can always be seen. They never disappear completely, though they do become fainter after more prolonged fixation. That the chambered structure cannot be seen in the living organism I do not regard as any proof of its non-existence. For the width of the cell is small (less than 2 μ) : the cell-membrane is fairly thick and possesses a considerable degree of refractivity : and the difference in refractivity between the protoplasm forming the walls of the chambers and that which fills them is probably not very great in the living organism. The chambered structure appears with such constancy in organisms fixed with osmic acid or corrosive sublimate that it will require a good deal more evidence than that furnished by Gross to prove that it does not exist.

Swellengrebel's (1907) original account of C. balbianii differs in some ways from his later description (1909), in which he records appearances which are consistent with my interpretations. The transverse bars of chromatin which he describes are, I believe, similar to the transverse bars which I have frequently seen, and are produced in precisely the same way—by imperfect fixation. Swellengrebel states that he fixed the organisms in formaldehyde (1907, p. 19), but he appears to have overlooked the fact that fixation in the way he describes is inadequate unless employed in conjunction with after-treatment with alcohol—a point which I have already had occasion to point out elsewhere (Dobell, 1910A).

It is apparent from the foregoing, therefore, that whereas Schellack appears to regard the body of a Cristispira as being chiefly composed of a nuclear structure, Gross regards it as enucleate, and I regard the nucleus as being in all probability represented by chromidial structures arranged in the manner described in previous pages (see p. 521).

Plasmolysis.—Swellengrebel (1909) has proved that

Cristispiræ are plasmolysable. The phenomenon is so often seen in organisms which have been dried, or are drying, in a drop of sea-water, that it is almost inconceivable that anyone should have stated that the organisms are implasmolysable. I think there can be no doubt whatever that plasmolysis may be caused in these organisms, and that it is similar to that which may be seen in many Bacteria.

Division.—My own conclusions regarding division are completely in accord with those of Schellack and Gross. Division is transverse, and not longitudinal. The errors of interpretation which have led many workers to believe that longitudinal division occurs have been fully discussed and elucidated by Gross. Further discussion of the matter therefore appears to me unnecessary.

Polarity.—A point of considerable importance, but one which has received hardly any attention from those who have discussed the affinities of the Cristispiræ and similar organisms, lies in connection with what I may term the "polarity" of the cell. All flagellate Protozoa possess an antero-posterior differentiation—that is to say, they show by their movements that one end of the body is the front end, the other the hind end. It is therefore correct to speak of their movements as backward or forward movements. The front end is usually the end which bears the flagellum. Now in the Bacteria no such differentiation can be observed. Spirilla and Bacilli cannot correctly be said to move backwards or forwards, because neither end is definitely differentiated as anterior or posterior. In other words, either end is a facultative anterior or posterior end.

In this respect Cristispira and the other so-called Spirochæts are similar to the Bacteria, and stand in sharp contrast with the flagellate Protozoa.

The point is not one to be ignored. For it is evident that a differentiation of this sort must involve the organisation of the whole organism, and must therefore be of profound significance.

Flexibility.—It has more than once been urged that

Cristispira and its allies, being flexible and not rigid organisms, show affinities with the Protozoa and not with the Bacteria in consequence. I do not know who is responsible for the original statement that all Bacteria are rigid organisms, but it is certain that such a statement cannot be accepted. Many Bacteria of large size are flexible to a considerable extent. I have shown this to be the case in Bacillus flexilis (Dobell, 1908) and a number of allied forms (Dobell, 1910A). It is therefore manifest that flexibility cannot be used as a criterion for judging whether the Spirochæts are to be ranked among Protozoa or Bacteria.

Conjugation.—The organisms described as "gametes" by Perrin and others, and the stages said by them to represent conjugation stages, are all quite arbitrarily so designated. I believe there is absolutely not a vestige of evidence that conjugation occurs in these organisms. Neither Swellengrebel, nor Schellack, nor Gross, nor myself could find any indication of sexual phenomena in this group. Both Schellack and Gross have discussed the matter more fully, and I am in complete agreement with their conclusions.

Encystment.—Whether Cristispiræ encyst or not is a point which is still undetermined. I believe the "cysts" described by Perrin and others are really to be regarded as the results of degeneration or plasmoptysis. Schellack and Gross both appear to be of the same opinion. At all events, it may be said with justice, I believe, that no clear case of encystment has yet been described in Cristispiræ.

Affinities and Classification.

Having now briefly noted the more important features in the structure and life-cycle of the Cristispiræ, it is possible to discuss the affinities and classification of these most remarkable organisms.

At the present moment it is customary to assemble under the common name "Spirochæts" three different groups of unicellular organisms. These are (1) the Cristispiræ,

parasitic in Lamellibranchs, (2) the much smaller parasitic organisms like "Spirochæta" pallida, "S." buccalis, the organisms of relapsing fevers, etc., (3) the free-living forms Spirochæta plicatilis and its allies.

Now the name Spirochæta[1] was introduced by Ehrenberg in 1833 for the free-living organism S. plicatilis. It must therefore be applied to this and similar organisms. The structure of S. plicatilis has been described by Schaudinn (1905, 1907). According to him there is an undulating membrane and a nucleus in the form of a longitudinal filament surrounded by chromidia—these two elements corresponding respectively to the kinetic and trophic nuclei of a trypanosome. Reproduction occurs by multiple transverse fission.

Quite recently these organisms have been more carefully studied by Zülzer (1910), whose observations differ greatly from those of Schaudinn. She interprets the axial filament as an elastic body—not part of the nucleus. The latter is represented by large, regularly arranged chromatin granules. There is no undulating membrane. If this description is correct,[2] it is obvious that S. plicatilis is a very different organism from Cristispira. Anyone who has observed living specimens of S. plicatilis would, I should think, be impressed by their dissimilarity to Cristispiræ—both as regards movements and general appearance. This, at all events, is my own impression. The bodies of both are flexible and spirally wound, but beyond this there is no great resemblance. The differences are at least sufficiently great to justify the bestowal of different generic names upon the two organisms. As Gross has introduced the name Cristispira for the mollusc Spirochæts it should henceforth be adopted.

The smaller parasitic Spirochæts—such as the syphilis

[1] The correct spelling of this name is Spirochæta, and not Spirochæte, as adopted by Doflein (1909) and numerous other writers.

[2] I have every reason to believe it is, as I had an opportunity of conversing with Frl. Dr. Zülzer and seeing some of her preparations at the International Zoological Congress in Graz this year (1910).

organism, the organisms of relapsing fevers, etc.—differ not a little from Cristispira and Spirochæta. In the forms which I have been able to study myself,[1] I have never been able to make out any definite structure—chiefly on account of their very small size. I believe that no protoplasmic structure similar to that of either S. plicatilis or Cristispira is visible. I also regard it as exceedingly doubtful that a crista is present. The method of division is, I believe, in all probability always transverse. Although the facies of these organisms is very similar to that of Cristispiræ, I think it is advisable to keep the two groups of organisms in separate genera for the present.

Regarding the generic name which must be applied to these organisms, it is obvious that as neither Spirochæta nor Cristispira can be used, some other name must be selected. The name Spironema, proposed by Vuillemin (1905) for the syphilis organism, is pre-occupied—having been used by Klebs for a flagellate. Schaudinn (1905A) therefore proposed the name Treponema—a name which must stand, according to the rules of nomenclature. If it be allowed that the small parasitic Spirochæts are similar to the syphilis organism,[2] it therefore follows that they must all be placed in the genus Treponema. It appears to me advisable to adopt this system.

For the three groups of organisms which are included in the common name "Spirochæts" there are therefore three generic names already in existence. On the assumption, then, that these three groups are sufficiently akin to one another to justify their being collected into a common class—an assumption which appears to me to be justified in our present state of knowledge—I propose to classify the Spirochæts as follows:

[1] These are especially forms from the gut of the frog and toad (Dobell, 1908), from termites (Dobell, 1910), and "S." buccalis and "S." dentium (unpublished observations).

[2] I see no valid reason for drawing a generic distinction between Treponema pallidum and such forms as "Spirochæta" recurrentis, "S." duttoni, "S." dentium, etc.

Spirochætoidea.

Genus 1.—Spirochæta Ehrenberg. Free-living forms, freshwater or marine. Examples: S. plicatilis Ehrenberg, S. gigantea Warming.

Genus 2.—Treponema Schaudinn. Parasitic in animals (Vertebrates and Invertebrates). Examples: T. pallidum Schaudinn, T. recurrentis Lebert, T. dentium Koch, T. gallinarum Blanchard, etc., etc.

Genus 3.—Cristispira Gross. Parasitic in Lamellibranchia. Examples: C. balbianii Certes, C. anodontæ Keysselitz, C. pectinis Gross, C. veneris, etc., etc.

The exact classificatory value to be attached to the group Spirochætoidea cannot at present be accurately determined. The name stands for a group of Protista which, like several other groups (e.g. Bacteria, Mycetozoa, Myxobacteria), cannot at present be regarded as a "class," "order," or any other sort of subdivision of another group, but must be regarded as an independent group of unicellular organisms which show very little affinity to any other group.

This last statement requires some further qualification. Many workers regard the Spirochæts as showing affinities to other Protista. It has been suggested that there are resemblances between them and the flagellate Protozoa, the Bacteria, and the Cyanophyceæ.

Schaudinn was the first to express the opinion that the Spirochæts are allied to the Trypanosomes, and hence to the flagellate Protozoa. Krzysztalowicz and Siedlecki (1908) go so far as to place them in a group Spirilloflagellata among the Mastigophora. Doflein (1909) places them in a group—Proflagellata—between the Bacteria and the Mastigophora. Now I think that I am completely justified—from what I have already pointed out in the preceding part of this paper—in stating that there is not one character of

importance which is common to Spirochæts and Flagellates—save that both are unicellular. It is, to me, most remarkable that anyone can see any real resemblance between a Spirochæt and a Trypanosome. The nuclear and cytoplasmic structures are wholly different: a Trypanosome has a flagellum, a Spirochæt has none:[1] the crista is not like an undulating membrane: the cell-membranes are not similar: and moreover, the method of division is quite different in the two groups of organisms. As regards conjugation, nothing has been proved either in Trypanosomes or Spirochæts, so that its occurrence or non-occurrence can furnish no grounds for discussion of affinities between the two groups. The flexibility of Spirochæts also, as I have pointed out, affords no criterion for determining their protozoal or bacterial affinities.

Many workers regard the Spirochæts as Bacteria. Novy and Knapp (1906) place them in the genus Spirillum. Swellengrebel (1907) places the Spirochæts and Spirilla in the same family (Spirillaceæ) among the Bacteria. Schellack (1909) suggests that the Spirochæts are related to the Cyanophyceæ by way of Spirulina and similar forms. Gross (1910) finally places Spirochæta with the Cyanophyceæ, and Cristispira and Treponema with the Bacteria. Zülzer (1910), however, who has made a special study of S. plicatilis and the spiral forms of Cyanophyceæ (Spirulina, Arthrospira), has shown that there is no real similarity between these organisms. Affinities between Spirochæts and Cyanophyceæ appear therefore not to exist.

Now beyond a certain superficial similarity of form between certain Spirochæts and Spirilla, there is really no reason for regarding Spirochæts as Bacteria. The points of similarity are chiefly these—both possess the same sort of cell polarity (see p. 532), both divide transversely, both are plasmolysable.

[1] The "flagella" of various species of Treponema are probably—as has often been pointed out already—merely the drawn-out ends of organisms which have just resulted from the transverse division of a longer organism. They have nothing to do with the flagella of Protozoa or Bacteria.

But the same might be said of many other Protista. Two most important characters of the Bacteria—the formation of endospores and the possession of flagella—are not encountered in the Spirochæts. The structure of the cell, especially as regards the nucleus, in Cristispira and Spirochæta is quite different from that of Spirilla. With regard to the latter, I would refer the reader to my work on the cytology of the Bacteria (Dobell, 1910A). There is, in fact, no real reason for regarding Spirochæts as Bacteria.

There seems to be a curious tendency on the part of many workers to reason thus: Spirochæts are not Protozoa, therefore they are Bacteria; or conversely, they are not Bacteria, therefore they are Protozoa. The premisses are both correct, I believe, but the deductions are both wrong. Spirochæts are neither Protozoa nor Bacteria; they are a group of Protista which stands alone. They certainly have a few characters in common with Bacteria, but the differences greatly outweigh these.

In conclusion, I will summarise the results to which my work has led me. They are as follows:

The organisms commonly called Spirochæts may be conveniently collected into a single group, for which I propose the name Spirochætoidea. This group comprises three different sets of forms, which may be correspondingly classified in three different genera — Spirochæta, Treponema, Cristispira. These three groups of organisms, whilst showing certain resemblances to one another, possess no definite relations with Protozoa, Bacteria, or Cyanophyceæ. The Spirochætoidea should therefore be regarded—for the present—as a group of Protista which stands apart.

Imperial College of Science and
Technology, London.
November, 1910.

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EXPLANATION OF PLATE 20,

Illustrating Mr. C. Clifford Dobell's paper "On Cristispira veneris nov. spec., and the Affinities and Classification of Spirochæts."

[All figures are drawn from stained preparations of Cristispira veneris n. sp., from the crystalline style of Venus (Meretrix) casta Chem., taken in Tamblegam Lake, E. Province, Ceylon. The drawings were made under a Zeiss 2 mm. apochromatic oil-immersion, with compensating oculars 6, 12, and 18. The magnification of the figures is approximately 2000 diameters.]

Fig. 1.—An average-sized individual, in optical section. The general form of the body is well seen. Note also the chambered structure of the cytoplasm and the arrangement of the purple-stained granules. (Osmic vapour 30 secs. absolute alcohol; Giemsa's stain.)

Fig. 2.—A somewhat extended individual, showing the disposition of the crista and the structure of the protoplasm. The body is seen in optical section, but the crista is shown as it appears when focussed carefully at different levels. (Osmic vapour [several minutes]; absolute alcohol, Giemsa.)

Fig. 3.—An individual which has been macerated in a drop of sea-water, allowed to dry, then fixed in absolute alcohol and stained by Giemsa's method. The organism has undergone plasmoptysis, and the crista shows a fibrillar structure.

Fig. 4.—A dividing organism in the stage of incurvation. (Osmic vapour, absolute alcohol, Giemsa.)

Fig. 5.—Part of an almost completely divided organism in incurvation stage. The upper end corresponds with the upper end of fig. 4, being the point at which fission occurs. (Slightly more highly magnified than the other figures.) (Osmic vapour, absolute alcohol, Giemsa.)

Fig. 6.—An individual which is almost completely divided into two. Stage following incurvation. At *a*, a darkly stained chamber. (Osmic vapour [several minutes], absolute alcohol, Giemsa.)

Fig. 7.—Middle region of an individual which is opening out after incurvation. Division of body and crista is seen. (Osmic vapour 30 secs., absolute alcohol, Giemsa.)

Fig. 8.—Part of the body of an organism which has been fixed by exposure for several minutes to osmic vapour, then treated with absolute alcohol, stained with Giemsa, and differentiated in alcohol. At *a* the dark blue granules are distinctly seen; at *b* is seen a thickened partition.

Fig. 9.—Part of another organism, treated like the preceding, but more deeply stained. The granules are not sharply differentiated from the walls of the chambers. At two points new chambers are being formed.

Fig. 10.—Part of a dried organism fixed in absolute alcohol and stained with Giemsa. "Nuclear" structures in the form of a spiral or zig-zag filament (*a*), a transverse bar (*b*), granules (*c*), a tetrad (*d*), etc., are seen.